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(21) International Application Number: PCT/US92/08787 (22) International Filing Date: 15 October 1992 (15.10.92) (30) Priority data: 798,392 26 November 1991 (26.11.91) US (71) Applicant: WARNER-LAMBERT COMPANY [US/US]; 201 Tabor Road, Morris Plains, NJ 07950 (US). (72) Inventor: MARTIN, Alain ; 31 Country Club Drive, Ringoes, NJ 08551 (US). (74) Agents: BULLITT, Richard, S. et al.; Warner-Lambert Company, 201 Tabor Road, Morris Plains, NJ 07950 (US).		(81) Designated States: AU, CA, JP, European patent (AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, SE). Published <i>With international search report.</i>
(54) Title: WOUND HEALING COMPOSITIONS CONTAINING A PYRUVATE, AN ANTIOXIDANT AND A MIXTURE OF FATTY ACIDS (57) Abstract The present invention pertains to therapeutic wound healing compositions. The compositions comprise (a) pyruvate selected from the group consisting of pyruvic acid, pharmaceutically acceptable salts of pyruvic acid, and mixtures thereof, (b) an antioxidant, and (c) a mixture of saturated and unsaturated fatty acids wherein the fatty acids are those fatty acids required for repair of cellular membranes and resuscitation of mammalian cells. The therapeutic compositions may be utilized in a wide variety of topical and ingestible pharmaceutical products. This invention also relates to methods for preparing and using the therapeutic compositions and the topical pharmaceutical products in which the therapeutic compositions may be used.		

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WOUND HEALING COMPOSITIONS CONTAINING A PYRUVATE, AN
ANTIOXIDANT AND A MIXTURE OF FATTY ACIDS

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BACKGROUND OF THE INVENTION

This application is a continuation-in-part of
application serial no. 663,500, filed 1 March 1991.

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1. Field of the Invention

This invention pertains to therapeutic wound
healing compositions useful for increasing the
proliferation and resuscitation rate of mammalian cells.
25 More particularly, the wound healing compositions
comprise (a) pyruvate, (b) an antioxidant, and (c) a
mixture of saturated and unsaturated fatty acids. This
invention also pertains to methods for preparing and
using the wound healing compositions and the topical
30 pharmaceutical products in which the therapeutic
compositions may be used.

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2. Description of the Background

Wounds are bodily injuries caused by physical
means which disrupt the normal continuity of structures.
Such bodily injuries include contusions, wounds in which

the skin is unbroken, incisions, wounds in which the skin is broken by a cutting instrument, and lacerations, wounds in which the skin is broken by a dull or blunt instrument. Wounds may be caused by accidents or by surgical procedures. Patients who suffer major wounds could benefit from an enhancement in the wound healing process.

Wound healing consists of a series of processes whereby injured tissue is repaired, specialized tissue is regenerated, and new tissue is reorganized into a scar. Wound healing consists of three major phases: a) an inflammation phase (0-3 days), b) a cellular proliferation phase (3-12 days), and (c) a remodeling phase (3 days-6 months).

During the inflammation phase, platelet aggregation and clotting form a matrix which traps plasma proteins and blood cells to induce the influx of various types of cells. During the cellular proliferation phase, new connective or granulation tissue and blood vessels are formed. During the remodeling phase, granulation tissue is replaced by a network of collagen and elastin fibers leading to the formation of scar tissue.

When cells are injured or killed as a result of a wound, a wound healing step is desirable to resuscitate the injured cells and produce new cells to replace the dead cells. The healing process requires the reversal of cytotoxicity, the suppression of inflammation, and the stimulation of cellular viability and proliferation. Wounds require low levels of oxygen in the initial stages of healing to suppress oxidative damage and higher levels of oxygen in the later stages of healing to promote collagen formation by fibroblasts.

Stressed and injured mammalian cells are exposed to activated oxygen species such as superoxide (O_2^-), hydrogen peroxide (H_2O_2), hydroxyl radical (OH^\cdot),

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and singlet oxygen ($^1\text{O}_2$). *In vivo*, these reactive oxygen intermediates are generated by cells in response to aerobic metabolism, catabolism of drugs and other xenobiotics, ultraviolet and x-ray radiation, and the respiratory burst of phagocytic cells (such as white blood cells) to kill invading bacteria such as those introduced through wounds. Hydrogen peroxide, for example, is produced during respiration of most living organisms especially by stressed and injured cells.

These active oxygen species can injure cells. An important example of such damage is lipid peroxidation which involves the oxidative degradation of unsaturated lipids. Lipid peroxidation is highly detrimental to membrane structure and function and can cause numerous cytopathological effects. Cells defend against lipid peroxidation by producing radical scavengers such as superoxide dismutase, catalase, and peroxidase. Injured cells have a decreased ability to produce radical scavengers. Excess hydrogen peroxide can react with DNA to cause backbone breakage, produce mutations, and alter and liberate bases. Hydrogen peroxide can also react with pyrimidines to open the 5, 6-double bond, which reaction inhibits the ability of pyrimidines to hydrogen bond to complementary bases, Hallaender et al. (1971). Such oxidative biochemical injury can result in the loss of cellular membrane integrity, reduced enzyme activity, changes in transport kinetics, changes in membrane lipid content, and leakage of potassium ions, amino acids, and other cellular material.

Antioxidants have been shown to inhibit damage associated with active oxygen species. For example, pyruvate and other alpha-ketoacids have been reported to react rapidly and stoichiometrically with hydrogen peroxide to protect cells from cytolytic effects, O'Donnell-Tormey et al., J. Exp. Med., 165, pp. 500-514 (1987).

United States patents nos. 3,920,835,
3,984,556, and 3,988,470, all issued to Van Scott et al.,
disclose methods for treating acne, dandruff, and palmar
keratosis, respectively, which consist of applying to the
5 affected area a topical composition comprising from about
1% to about 20% of a lower aliphatic compound containing
from two to six carbon atoms selected from the group
consisting of alpha-hydroxyacids, alpha-ketoacids and
esters thereof, and 3-hydroxybutyric acid in a
10 pharmaceutically acceptable carrier. The aliphatic
compounds include pyruvic acid and lactic acid.

United States patents nos. 4,105,783 and
4,197,316, both issued to Yu et al., disclose a method
15 and composition, respectively, for treating dry skin
which consists of applying to the affected area a topical
composition comprising from about 1% to about 20% of a
compound selected from the group consisting of amides and
ammonium salts of alpha-hydroxyacids, beta-hydroxyacids,
20 and alpha-ketoacids in a pharmaceutically acceptable
carrier. The compounds include the amides and ammonium
salts of pyruvic acid and lactic acid.

United States patent no. 4,234,599, issued to
25 Van Scott et al., discloses a method for treating actinic
and nonactinic skin keratoses which consists of applying
to the affected area a topical composition comprising an
effective amount of a compound selected from the group
consisting of alpha-hydroxyacids, beta-hydroxyacids, and
30 alpha-ketoacids in a pharmaceutically acceptable carrier.
The acidic compounds include pyruvic acid and lactic
acid.

United States patent no. 4,294,852, issued to
35 Wildnauer et al., discloses a composition for treating
skin which comprises the alpha-hydroxyacids, beta-
hydroxyacids, and alpha-ketoacids disclosed above by
Van Scott et al. in combination with C₃-C₈ aliphatic
alcohols.

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United States patent no. 4,663,166, issued to Veech, discloses an electrolyte solution which comprises a mixture of L-lactate and pyruvate in a ratio from 20:1 to 1:1, respectively, or a mixture of D-beta-hydroxybutyrate and acetoacetate, in a ratio from 6:1 to 0.5:1, respectively.

Sodium pyruvate has been reported to reduce the number of erosions, ulcers, and hemorrhages on the gastric mucosa in guinea pigs and rats caused by acetylsalicylic acid. The analgesic and antipyretic properties of acetylsalicylic acid were not impaired by sodium pyruvate, Puschmann, Arzneimittelforschung, 33, pp. 410-415 and 415-416 (1983).

Pyruvate has been reported to exert a positive inotropic effect in stunned myocardium, which is a prolonged ventricular dysfunction following brief periods of coronary artery occlusions which does not produce irreversible damage, Mentzer et al., Ann. Surg., 209, pp. 629-633 (1989).

Pyruvate has been reported to produce a relative stabilization of left ventricular pressure and work parameter and to reduce the size of infarctions. Pyruvate improves resumption of spontaneous beating of the heart and restoration of normal rates and pressure development, Bunger et al., J. Mol. Cell. Cardiol., 18, pp. 423-438 (1986), Mochizuki et al., J. Physiol. (Paris), 76, pp. 805-812 (1980), Regitz et al., Cardiovasc. Res., 15, pp. 652-658 (1981), Giannelli et al., Ann. Thorac. Surg., 21, pp. 386-396 (1976).

Sodium pyruvate has been reported to act as an antagonist to cyanide intoxicification (presumably through the formation of a cyanohydrin) and to protect against the lethal effects of sodium sulfide and to retard the

onset and development of functional, morphological, and biochemical measures of acrylamide neuropathy of axons, Schwartz et al., Toxicol. Appl. Pharmacol., 50, pp. 437-442 (1979), Sabri et al., Brain Res., 483, pp. 1-11 (1989).

A chemotherapeutic cure of advanced L1210 leukemia has been reported using sodium pyruvate to restore abnormally deformed red blood cells to normal. The deformed red blood cells prevented adequate drug delivery to tumor cells, Cohen, Cancer Chemother. Pharmacol., 5, pp. 175-179 (1981).

Primary cultures of heterotopic tracheal transplant exposed *in vivo* to 7, 12-dimethylbenz(a)anthracene were reported to be successfully maintained in enrichment medium supplemented with sodium pyruvate along with cultures of interleukin-2 stimulated peripheral blood lymphocytes, and plasmacytomas and hybridomas, pig embryos, and human blastocysts, Shacter, J. Immunol. Methods, 99, pp. 259-270 (1987), Marchok et al., Cancer Res., 37, pp. 1811-1821 (1977), Davis, J. Reprod. Fertil. Suppl., 33, pp. 115-124 (1985), Okamoto et al., No To Shinkei, 38, pp. 593-598 (1986), Cohen et al., J. In Vitro Fert. Embryo Transfer, 2, pp. 59-64 (1985).

United States patents nos. 4,158,057, 4,351,835, 4,415,576, and 4,645,764, all issued to Stanko, disclose methods for preventing the accumulation of fat in the liver of a mammal due to the ingestion of alcohol, for controlling weight in a mammal, for inhibiting body fat while increasing protein concentration in a mammal, and for controlling the deposition of body fat in a living being, respectively. The methods comprise administering to the mammal a therapeutic mixture of pyruvate and dihydroxyacetone, and optionally riboflavin. United States patent no. 4,548,937, issued to Stanko, discloses a method for

controlling the weight gain of a mammal which comprises administering to the mammal a therapeutically effective amount of pyruvate, and optionally riboflavin. United States patent no. 4,812,479, issued to Stanko, discloses a method for controlling the weight gain of a mammal which comprises administering to the mammal a therapeutically effective amount of dihydroxyacetone, and optionally riboflavin and pyruvate.

Rats fed a calcium-oxalate lithogenic diet including sodium pyruvate were reported to develop fewer urinary calculi (stones) than control rats not given sodium pyruvate, Ogawa et al., Hinyokika Kyo, 32, pp. 1341-1347 (1986).

United States patent no. 4,521,375, issued to Houlsby, discloses a method for sterilizing surfaces which come into contact with living tissue. The method comprises sterilizing the surface with aqueous hydrogen peroxide and then neutralizing the surface with pyruvic acid.

United States patent no. 4,416,982, issued to Tauda et al., discloses a method for decomposing hydrogen peroxide by reacting the hydrogen peroxide with a phenol or aniline derivative in the presence of peroxidase.

United States patent no. 4,696,917, issued to Lindstrom et al., discloses an eye irrigation solution which comprises Eagle's Minimum Essential Medium with Earle's salts, chondroitin sulfate, a buffer solution, 2-mercaptoethanol, and a pyruvate. The irrigation solution may optionally contain ascorbic acid and alpha-tocopherol. United States patent no. 4,725,586, issued to Lindstrom et al., discloses an irrigation solution which comprises a balanced salt solution, chondroitin sulfate, a buffer solution, 2-mercaptoethanol, sodium bicarbonate or dextrose, a pyruvate, a sodium phosphate

buffer system, and cystine. The irrigation solution may optionally contain ascorbic acid and gamma-tocopherol.

5 United States patent no. 3,887,702 issued to Baldwin, discloses a composition for treating fingernails and toenails which consists essentially of soybean oil or sunflower oil in combination with Vitamin E.

10 United States patent no. 4,847,069, issued to Bissett et al., discloses a photoprotective composition comprising (a) a sorbohydroxamic acid, (b) an anti-inflammatory agent selected from steroidal anti-inflammatory agents and a natural anti-inflammatory agent, and (c) a topical carrier. Fatty acids may be
15 present as an emollient. United States patent no. 4,847,071, issued to Bissett et al., discloses a photoprotective composition comprising (a) a tocopherol or tocopherol ester radical scavenger, (b) an anti-inflammatory agent selected from steroidal anti-inflammatory agents and a natural anti-inflammatory agent, and (c) a topical carrier. United States patent
20 no. 4,847,072, issued to Bissett et al., discloses a topical composition comprising not more than 25% tocopherol sorbate in a topical carrier.

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United States patent no. 4,533,637, issued to Yamane et al., discloses a culture medium which comprises a carbon source, a nucleic acid source precursor, amino acids, vitamins, minerals, a lipophilic nutrient, and
30 serum albumin, and cyclodextrins. The lipophilic substances include unsaturated fatty acids and lipophilic vitamins such as Vitamin A, D, and E. Ascorbic acid may also be present.

35 United Kingdom patent application no. 2,196,348A, to Kovar et al., discloses a synthetic culture medium which comprises inorganic salts, monosaccharides, amino acids, vitamins, buffering agents, and optionally sodium pyruvate adding magnesium hydroxide

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or magnesium oxide to the emulsion. The oil phase may include chicken fat.

5 United States patent no. 4,284,630, issued to Yu et al., discloses a method for stabilizing a water-in-oil emulsion which comprises adding magnesium hydroxide or magnesium oxide to the emulsion. The oil phase may include chicken fat.

10 Preparation-HTM has been reported to increase the rate of wound healing in artificially created rectal ulcers. The active ingredients in Preparation-HTM are skin respiratory factor and shark liver oil, Subramanyam et al., Digestive Diseases and Sciences, 29,
15 pp. 829-832 (1984).

The addition of sodium pyruvate to bacterial and yeast systems has been reported to inhibit hydrogen peroxide production, enhance growth, and protect the
20 systems against the toxicity of reactive oxygen intermediates. The unsaturated fatty acids and saturated fatty acids contained within chicken fat enhanced membrane repair and reduced cytotoxicity. The antioxidants glutathione and thioglycollate reduced the
25 injury induced by oxygen radical species, Martin, Ph.D. thesis, (1987-89).

United States patent no. 4,615,697, issued to Robinson, discloses a controlled release treatment
30 composition comprising a treating agent and a bioadhesive agent comprising a water-swellaable but water-insoluble, fibrous cross-linked carboxy-functional polymer.

European patent application no. 0410696A1, to
35 Kellaway et al., discloses a mucoadhesive delivery system comprising a treating agent and a polyacrylic acid cross-linked with from about 1% to about 20% by weight of a polyhydroxy compound such as a sugar, cyclitol, or lower polyhydric alcohol.

While the above therapeutic compositions are reported to inhibit the production of reactive oxygen intermediates, none of the above compositions are entirely satisfactory wound healing compositions. None of the compositions has the ability to simultaneously decrease cellular levels of hydrogen peroxide production, increase cellular resistance to cytotoxic agents, increase rates of cellular proliferation, and increase cellular viability to protect and resuscitate mammalian cells. The present invention provides such improved therapeutic wound healing compositions without the disadvantages characteristic of previously known compositions.

SUMMARY OF THE INVENTION

The present invention pertains to therapeutic wound healing compositions. The compositions comprise (a) pyruvate selected from the group consisting of pyruvic acid, pharmaceutically acceptable salts of pyruvic acid, and mixtures thereof, (b) an antioxidant, and (c) a mixture of saturated and unsaturated fatty acids wherein the fatty acids are those fatty acids required for the repair of cellular membranes and resuscitation of mammalian cells. The therapeutic compositions may be utilized in a wide variety of topical and ingestible pharmaceutical products. This invention also relates to methods for preparing and using the therapeutic compositions and the topical pharmaceutical products in which the therapeutic compositions may be used.

BRIEF DESCRIPTION OF THE FIGURES

FIGURE 1 is a photograph of wounded mice after 4 days of treatment with: preparation HTM (Example A); a petrolatum base formulation containing live yeast cell derivative, shark oil, and a mixture of sodium pyruvate, vitamin E, and chicken fat (Example B); a petrolatum base formulation containing live yeast cell derivative and shark oil (Example C); and no composition (Example E, control).

FIGURE 2 is a photograph of a wounded mouse after 4 days of treatment with a petrolatum base formulation only (Example D).

DETAILED DESCRIPTION OF THE INVENTION

Applicant has discovered therapeutic wound healing compositions for increasing the resuscitation rate of injured mammalian cells and the proliferation rate of new mammalian cells to replace dead cells. Cells treated with the therapeutic compositions of the present invention show decreased levels of hydrogen peroxide production, increased resistance to cytotoxic agents, increased rates of proliferation, and increased viability. Wounded mammals treated with the therapeutic compositions show significantly improved wound closing and healing over untreated mammals and mammals treated with conventional healing compositions.

The term "injured cell" as used herein means a cell which has (a) injured membranes so that transport through the membranes is diminished resulting in an increase in toxins and normal cellular wastes inside the cell and a decrease in nutrients and other components necessary for cellular repair inside the cell, (b) an

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increase in concentration of oxygen radicals inside the cell because of the decreased ability of the cell to produce antioxidants and enzymes, and (c) damaged DNA, RNA, and ribosomes which must be repaired or replaced before normal cellular functions can be resumed. The term "resuscitation" of injured mammalian cells as used herein means the reversal of cytotoxicity, the stabilization of the cellular membrane, an increase in the proliferation rate of the cell, and/or the normalization of cellular functions such as the secretion of growth factors, hormones, and the like. The term "cytotoxicity" as used herein means a condition caused by a cytotoxic agent that injures the cell. Injured cells do not proliferate because injured cells expend all energy on cellular repair. Aiding cellular repair promotes cellular proliferation.

Epidermal keratinocytic cells and monocytic cells have multiple oxygen generating mechanisms and the degree to which each type of mechanism functions differs in each type of cell. In monocytes, for example, the respiratory bursting process is more pronounced than in epidermal keratinocytes. Hence, the components in the therapeutic compositions of the present invention may vary depending upon the types of cells involved in the condition being treated.

In a first embodiment, the therapeutic wound healing composition for treating mammalian cells, preferably epidermal keratinocytes, comprises (a) pyruvate, (b) an antioxidant, and (c) a mixture of saturated and unsaturated fatty acids.

While not wishing to be bound by theory, applicant believes that pyruvate (or pyruvic acid) can be transported inside a cell where it can act as an antioxidant to neutralize oxygen radicals in the cell. Pyruvate can also be used inside the cell in the citric acid cycle to provide energy to increase cellular

viability, and as a precursor in the synthesis of important biomolecules to promote cellular proliferation. In addition, pyruvate can be used in the multifunction oxidase system to reverse cytotoxicity. Antioxidants, especially lipid-soluble antioxidants, can be absorbed into the cell membrane to neutralize oxygen radicals and thereby protect the membrane. The combination of pyruvate inside the cell and an antioxidant in the cellular membrane functions in a synergistic manner to reduce hydrogen peroxide production in the cell to levels lower than can be achieved by use of either type of component alone.

The saturated and unsaturated fatty acids in the present invention are those fatty acids required for the repair of cellular membranes and resuscitation of mammalian cells. Hence, the fatty acids in the therapeutic composition, which may be in the form of mono-, di-, and/or triglycerides or free fatty acids, are readily available for the repair of injured cells and the production of new cells to replace dead cells. Cells injured by oxygen radicals need to produce unsaturated fatty acids to repair cellular membranes. However, the production of unsaturated fatty acids by cells requires oxygen. Thus, the injured cell needs high levels of oxygen to produce unsaturated fatty acids and at the same time needs to reduce the level of oxygen within the cell to reduce oxidative injury. By providing the cell with the unsaturated fatty acids needed for repair, the need of the cell to produce unsaturated fatty acids is reduced and the need for high oxygen levels is also reduced. The presence of mixtures of saturated and unsaturated fatty acids in the therapeutic composition significantly enhances the ability of pyruvate and the antioxidant to inhibit reactive oxygen production. By stabilizing the cellular membrane, unsaturated fatty acids also improve membrane function and enhance pyruvate transport into the cell. By improving the viability of the cells, unsaturated fatty acids also improve the repair of

cellular membranes rate of the cells. Hence, the three components in the therapeutic composition function together in a synergistic manner to increase the resuscitation rate of injured mammalian cells and the production of new cells.

In a second embodiment, the therapeutic wound healing composition for treating mammalian cells, preferably epidermal keratinocytes, comprises (a) pyruvate, (b) lactate, and (c) a mixture of saturated and unsaturated fatty acids. In this embodiment, lactate is employed instead of an antioxidant. Antioxidants react with, and neutralize, oxygen radicals after the radicals are already formed. Lactate, on the other hand, is a component in the cellular feedback mechanism and inhibits the respiratory bursting process to suppress the production of active oxygen species. The combination of pyruvate to neutralize active oxygen species and lactate to suppress the respiratory bursting process functions in a synergistic manner to reduce hydrogen peroxide production in the cell to levels lower than can be achieved by use of either type of component alone. The presence of mixtures of saturated and unsaturated fatty acids in the therapeutic composition significantly enhances the ability of pyruvate and lactate to inhibit reactive oxygen production. Hence, the three components in the therapeutic composition in this embodiment function together in a synergistic manner to increase the proliferation and resuscitation rate of mammalian cells.

In a third embodiment, the therapeutic wound healing composition for treating mammalian cells, preferably epidermal keratinocytes, comprises (a) an antioxidant, and (b) a mixture of saturated and unsaturated fatty acids. The presence of mixtures of saturated and unsaturated fatty acids in the therapeutic composition in this embodiment significantly enhances the ability of the antioxidant to inhibit reactive oxygen production. The combination of an antioxidant to

neutralize active oxygen species and fatty acids to
rebuild cellular membranes and reduce the need of the
cell for oxygen functions in a synergistic manner to
reduce hydrogen peroxide production in the cell to levels
5 lower than can be achieved by either type of component
alone. Hence, the components in the therapeutic
composition in this embodiment function together in a
synergistic manner to increase the proliferation and
resuscitation rate of mammalian cells.

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In a fourth embodiment, the therapeutic wound
healing composition for treating mammalian cells,
preferably monocytes, comprises (a) lactate, (b) an
antioxidant, and (c) a mixture of saturated and
15 unsaturated fatty acids. In this embodiment, lactate is
employed because the respiratory bursting process is more
pronounced in monocytes than in epidermal keratinocytes.
The combination of lactate to suppress the respiratory
bursting process and an antioxidant to neutralize active
20 oxygen species functions in a synergistic manner to
reduce hydrogen peroxide production in the cell to levels
lower than can be achieved by either component alone.
The presence of mixtures of saturated and unsaturated
fatty acids in the therapeutic composition in this
25 embodiment significantly enhances the ability of lactate
and the antioxidant to inhibit reactive oxygen
production. Hence, the three components in the
therapeutic composition in this embodiment function
together in a synergistic manner to increase the
30 proliferation and resuscitation rate of mammalian cells.

Accordingly, the combination of ingredients set
out in the above embodiments functions together in an
enhanced manner to increase the proliferation and
35 resuscitation rate of mammalian cells. The therapeutic
effect of the combination of the components in each of
the above embodiments is markedly greater than that
expected by the mere addition of the individual
therapeutic components. Hence, applicant's therapeutic

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wound healing compositions have the ability to decrease
intracellular levels of hydrogen peroxide production,
increase cellular resistance to cytotoxic agents,
increase rates of cellular proliferation, and increase
5 cellular viability.

The cells which may be treated with the
therapeutic compositions in the present invention are
mammalian cells. Although applicant will describe the
10 present therapeutic compositions as useful for treating
mammalian epidermal keratinocytes and mammalian
monocytes, applicant contemplates that all mammalian
cells which may be protected or resuscitated by
applicant's therapeutic compositions may be used in the
15 present invention. Keratinocytes are representative of
normal mammalian cells and are the fastest proliferating
cells in the body. The correlation between the reaction
of keratinocytes to injury and therapy and that of
mammalian cells in general is very high. Monocytes are
20 representative of specialized mammalian cells such as the
white blood cells in the immune system and the organ
cells in liver, kidney, heart, and brain. The mammalian
cells may be treated *in vivo* and *in vitro*.

25 Epidermal keratinocytes are the specialized
epithelial cells of the epidermis which synthesize
keratin, a scleroprotein which is the principal
constituent of epidermis, hair, nails, horny tissue, and
the organic matrix of the enamel of teeth. Mammalian
30 epidermal keratinocytes constitute about 95% of the
epidermal cells and together with melanocytes form the
binary system of the epidermis. In its various
successive stages, epidermal keratinocytes are also known
as basal cells, prickle cells, and granular cells.

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Monocytes are mononuclear phagocytic leukocytes
which undergo respiratory bursting and are involved in
reactive oxygen mediated damage within the epidermis.
Leukocytes are white blood cells or corpuscles which may

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be classified into two main groups: granular leukocytes (granulocytes) which are leukocytes with abundant granules in the cytoplasm and nongranular leukocytes (nongranulocytes) which are leukocytes without specific granules in the cytoplasm and which include the lymphocytes and monocytes. Phagocyte cells are cells which ingest microorganisms or other cells and foreign particles. Monocytes are also known as large mononuclear leukocytes, and hyaline or transitional leukocytes.

Pyruvic acid (2-oxopropionic acid, alpha-ketopropionic acid, CH_3COCOOH) or pyruvate (at physiological pH) is a fundamental intermediate in protein and carbohydrate metabolism and in the citric acid cycle. The citric acid cycle (tricarboxylic acid cycle, Krebs' cycle) is the major reaction sequence which executes the reduction of oxygen to generate adenosine triphosphate (ATP) by oxidizing organic compounds in respiring tissues to provide electrons to the transport system. Acetyl coenzyme A ("active acetyl") is oxidized in this process and is thereafter utilized in a variety of biological processes and is a precursor in the biosynthesis of many fatty acids and sterols. The two major sources of acetyl coenzyme A are derived from the metabolism of glucose and fatty acids. Glycolysis consists of a series of transformations wherein each glucose molecule is transformed in the cellular cytoplasm into two molecules of pyruvic acid. Pyruvic acid may then enter the mitochondria where it is oxidized by coenzyme A in the presence of enzymes and cofactors to acetyl coenzyme A. Acetyl coenzyme A can then enter the citric acid cycle.

In muscle, pyruvic acid (derived from glycogen) is reduced to lactic acid during exertion. Lactic acid is reoxidized and partially retransformed to glycogen during rest. Pyruvate can also act as an antioxidant to

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neutralize oxygen radicals in the cell and can be used in the multifunction oxidase system to reverse cytotoxicity.

5 The pyruvate in the present invention may be selected from the group consisting of pyruvic acid, pharmaceutically acceptable salts of pyruvic acid, and mixtures thereof. In general, the pharmaceutically acceptable salts of pyruvic acid may be alkali salts and alkaline earth salts. Preferably, the pyruvate is
10 selected from the group consisting of pyruvic acid, sodium pyruvate, potassium pyruvate, magnesium pyruvate, calcium pyruvate, zinc pyruvate, manganese pyruvate, and mixtures thereof. More preferably, the pyruvate is selected from the group of salts consisting of sodium
15 pyruvate, potassium pyruvate, magnesium pyruvate, calcium pyruvate, zinc pyruvate, manganese pyruvate, and mixtures thereof. Most preferably, the pyruvate is sodium pyruvate.

20 The amount of pyruvate present in the therapeutic compositions of the present invention is a therapeutically effective amount. A therapeutically effective amount of pyruvate is that amount of pyruvate necessary to increase the proliferation and resuscitation
25 rate of mammalian cells. The exact amount of pyruvate is a matter of preference subject to such factors as the type of condition being treated as well as the other ingredients in the composition. In a preferred embodiment, pyruvate is present in the therapeutic
30 composition in an amount from about 10% to about 50%, preferably from about 20% to about 45%, and more preferably from about 25% to about 40%, by weight of the therapeutic composition.

35 L-Lactic acid ((S)-2-hydroxypropanoic acid, (+) alpha-hydroxypropionic acid, $\text{CH}_3\text{CHOHCOOH}$) or lactate occurs in small quantities in the blood and muscle fluid of mammals. Lactic acid concentration increases in muscle and blood after vigorous activity. Lactate is a

component in the cellular feedback mechanism and inhibits the natural respiratory bursting process of cells thereby suppressing the production of oxygen radicals.

5 The lactate in the present invention may be selected from the group consisting of lactic acid, pharmaceutically acceptable salts of lactic acid, and mixtures thereof. In general, the pharmaceutically acceptable salts of lactic acid may be alkali salts and
10 alkaline earth salts. Preferably, the lactate is selected from the group consisting of lactic acid, sodium lactate, potassium lactate, magnesium lactate, calcium lactate, zinc lactate, manganese lactate, and mixtures thereof. More preferably, the lactate is selected from
15 the group consisting of lactic acid, sodium lactate, potassium lactate, magnesium lactate, calcium lactate, zinc lactate, manganese lactate, and mixtures thereof. Most preferably, the lactate is lactic acid.

20 The amount of lactate present in the therapeutic compositions of the present invention is a therapeutically effective amount. A therapeutically effective amount of lactate is that amount of lactate necessary to increase the proliferation and resuscitation
25 rate of mammalian cells. For a composition, a therapeutically effective amount of lactate is that amount necessary to suppress the respiratory bursting process of white blood cells to protect and resuscitate the mammalian cells. In general, a therapeutically
30 effective amount of lactate in a composition is from about 5 to about 10 times the amount of lactate normally found in serum. The exact amount of lactate is a matter of preference subject to such factors as the type of condition being treated as well as the other ingredients
35 in the composition. In a preferred embodiment, lactate is present in the therapeutic composition in an amount from about 10% to about 50%, preferably from about 20% to about 45%, and more preferably from about 25% to about 40%, by weight of the therapeutic composition.

Antioxidants are substances which inhibit oxidation or suppress reactions promoted by oxygen or peroxides. Antioxidants, especially lipid-soluble antioxidants, can be absorbed into the cellular membrane to neutralize oxygen radicals and thereby protect the membrane. The antioxidants useful in the present invention may be selected from the group consisting of Vitamin A (retinol), Vitamin A₂ (3, 4-didehydroretinol), all forms of carotene such as alpha-carotene, beta-carotene (beta, beta-carotene), gamma-carotene, delta-carotene, Vitamin C (ascorbic acid, L-ascorbic acid), all forms of tocopherol such as Vitamin E (alpha-tocopherol, 3,4-dihydro-2,5,7,8-tetramethyl-2-(4,8,12-trimethyltridecyl)-2H-1-benzopyran-6-ol), beta-tocopherol, gamma-tocopherol, and delta-tocopherol, and mixtures thereof. Preferably, the antioxidant is selected from the group of lipid-soluble antioxidants consisting of Vitamin A, beta-carotene, Vitamin E, and mixtures thereof. More preferably, the antioxidant is Vitamin E.

The amount of antioxidant present in the therapeutic compositions of the present invention is a therapeutically effective amount. A therapeutically effective amount of antioxidant is that amount of antioxidant necessary to increase the proliferation and resuscitation rate of mammalian cells. The exact amount of antioxidant is a matter of preference subject to such factors as the type of condition being treated as well as the other ingredients in the composition. In a preferred embodiment, the antioxidant is present in the therapeutic composition in an amount from about 10% to about 50%, preferably from about 20% to about 45%, and more preferably from about 25% to about 40%, by weight of the therapeutic composition.

The mixture of saturated and unsaturated fatty acids in the present invention are those fatty acids required for the repair of mammalian cellular membranes

and the production of new cells^{d/}. Hence, the fatty acids are readily incorporated into the cell and are immediately available for the repair of injured cells and the proliferation of new cells. By providing the cell with the unsaturated fatty acids needed for repair, the need of the cell for unsaturated fatty acids is reduced and the need for high oxygen levels is also reduced. Accordingly, the presence of the mixtures of saturated and unsaturated fatty acids in the therapeutic compositions significantly enhances the ability of pyruvate, lactate, and the antioxidant to inhibit reactive oxygen production.

Fatty acids are carboxylic acid compounds found in animal and vegetable fat and oil. Fatty acids are classified as lipids and are composed of chains of alkyl groups containing from 4 to 22 carbon atoms and 0-3 double bonds and characterized by a terminal carboxyl group, -COOH . Fatty acids may be saturated or unsaturated and may be solid, semisolid, or liquid. The most common saturated fatty acids are butyric acid (C_4), lauric acid (C_{12}), palmitic acid (C_{16}), and stearic acid (C_{18}). Unsaturated fatty acids are usually derived from vegetables and consist of alkyl chains containing from 16 to 22 carbon atoms and 0-3 double bonds with the characteristic terminal carboxyl group. The most common unsaturated fatty acids are oleic acid, linoleic acid, and linolenic acid (all C_{18} acids).

In general, the mixture of saturated and unsaturated fatty acids required for the repair of mammalian cellular membranes in the present invention may be derived from animal fats and waxes. Cells produce the chemical components and the energy required for cellular viability and store excess energy in the form of fat. Fat is adipose tissue stored between organs of the body to furnish a reserve supply of energy. The preferred animal fats and waxes have a fatty acid composition similar to that of human fat and the fat contained in

human breast milk. The preferred animal fats and waxes may be selected from the group consisting of human fat, chicken fat, cow fat (defined herein as a bovine domestic animal regardless of sex or age), sheep fat, horse fat, pig fat, and whale fat. The more preferred animal fats and waxes may be selected from the group consisting of human fat and chicken fat. The most preferred animal fat is human fat. Mixtures of other fats and waxes, such as vegetable waxes, marine oils (especially shark liver oil), and synthetic waxes and oils, which have a fatty acid composition similar to that of animal fats and waxes, and preferably to that of human fats and waxes, may also be employed. The mixture of saturated and unsaturated fatty acids may also be derived from animal and vegetable fats and waxes, and mixtures thereof.

In a preferred embodiment, the mixture of saturated and unsaturated fatty acids has a composition similar to that of human fat and comprises the following fatty acids: butyric acid, caproic acid, caprylic acid, capric acid, lauric acid, myristic acid, myristoleic acid, palmitic acid, palmitoleic acid, stearic, oleic acid, linoleic acid, linolenic acid, arachidic acid, and gaddoleic acid. Preferably, butyric acid, caproic acid, caprylic acid, capric acid, lauric acid, myristic acid, myristoleic acid, palmitic acid, palmitoleic acid, stearic, oleic acid, linoleic acid, linolenic acid, arachidic acid, and gaddoleic acid are present in the mixture in about the following percentages by weight, respectively (carbon chain number and number of unsaturations are shown parenthetically, respectively): 0.2%-0.4% (C_4), 0.1% (C_6), 0.3%-0.8% (C_8), 2.2%-3.5% (C_{10}), 0.9%-5.5% (C_{12}), 2.8%-8.5% (C_{14}), 0.1%-0.6% ($C_{14:1}$), 23.2%-24.6% (C_{16}), 1.8%-3.0% ($C_{16:1}$), 6.9%-9.9% (C_{18}), 36.0%-36.5% ($C_{18:1}$), 20%-20.6% ($C_{18:2}$), 7.5-7.8% ($C_{18:3}$), 1.1%-4.9% (C_{20}), and 3.3%-6.4% ($C_{20:1}$).

In another preferred embodiment, the mixture of saturated and unsaturated fatty acids is typically

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chicken fat comprising the following fatty acids: lauric acid, myristic acid, myristoleic acid, pentadecanoic acid, palmitic acid, palmitoleic acid, margaric acid, margaroleic acid, stearic, oleic acid, linoleic acid, linolenic acid, arachidic acid, and gaddoleic acid. Preferably, lauric acid, myristic acid, myristoleic acid, pentadecanoic acid, palmitic acid, palmitoleic acid, margaric acid, margaroleic acid, stearic, oleic acid, linoleic acid, linolenic acid, arachidic acid, and gaddoleic acid are present in the mixture in about the following percentages by weight, respectively: 0.1% (C₁₂), 0.8% (C₁₄), 0.2% (C_{14:1}), 0.1% (C₁₅), 25.3% (C₁₆), 7.2% (C_{16:1}), 0.1% (C₁₇), 0.1% (C_{17:1}), 6.5% (C₁₈), 37.7% (C_{18:1}), 20.6% (C_{18:2}), 0.8% (C_{18:3}), 0.2% (C₂₀), and 0.3% (C_{20:1}), all percentages +/- 10%.

The above fatty acids and percentages thereof present in the fatty acid mixture are given as an example. The exact type of fatty acid present in the fatty acid mixture and the exact amount of fatty acid employed in the fatty acid mixture may be varied in order to obtain the result desired in the final product and such variations are now within the capabilities of those skilled in the art without the need for undue experimentation.

The amount of fatty acids present in the therapeutic compositions of the present invention is a therapeutically effective amount. A therapeutically effective amount of fatty acids is that amount of fatty acids necessary to increase the repair of cellular membranes and resuscitation rate of mammalian cells. The exact amount of fatty acids employed is subject to such factors as the type and distribution of fatty acids employed in the mixture, the type of condition being treated, and the other ingredients in the composition. In a preferred embodiment, the fatty acids are present in the therapeutic composition in an amount from about 10% to about 50%, preferably from about 20% to about 45%, and

more preferably from about 25% to about 40%, by weight of the therapeutic composition.

5 In accord with the present invention, the therapeutic wound healing compositions for treating mammalian cells may be selected from the group consisting of:

10 (1) (a) pyruvate selected from the group consisting of pyruvic acid, pharmaceutically acceptable salts of pyruvic acid, and mixtures thereof;

(b) an antioxidant; and

15 (c) a mixture of saturated and unsaturated fatty acids wherein the fatty acids are those fatty acids required for the repair of cellular membranes and resuscitation of mammalian cells;

(2) (a) pyruvate selected from the group consisting of pyruvic acid, pharmaceutically acceptable salts of pyruvic acid, and mixtures thereof;

20 (b) lactate selected from the group consisting of lactic acid, pharmaceutically acceptable salts of lactic acid, and mixtures thereof; and

25 (c) a mixture of saturated and unsaturated fatty acids wherein the fatty acids are those fatty acids required for the repair of cellular membranes and resuscitation of mammalian cells;

(3) (a) an antioxidant; and

30 (b) a mixture of saturated and unsaturated fatty acids wherein the fatty acids are those fatty acids required for the repair of cellular membranes and resuscitation of mammalian cells;

(4) (a) lactate selected from the group consisting of lactic acid, pharmaceutically acceptable salts of lactic acid, and mixtures thereof;

(b) an antioxidant; and

35 (c) a mixture of saturated and unsaturated fatty acids wherein the fatty acids are those fatty acids required for the repair of cellular membranes and resuscitation of mammalian cells.

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In a preferred embodiment, the wound healing compositions for treating mammalian cells, preferably epidermal keratinocytes, may be selected from the group consisting of:

- 5 (1) (a) pyruvate selected from the group consisting of pyruvic acid, pharmaceutically acceptable salts of pyruvic acid, and mixtures thereof;
- (b) an antioxidant; and
- (c) a mixture of saturated and unsaturated
- 10 fatty acids wherein the fatty acids are those fatty acids required for the repair of cellular membranes and resuscitation of mammalian cells;
- (2) (a) pyruvate selected from the group consisting of pyruvic acid, pharmaceutically acceptable
- 15 salts of pyruvic acid, and mixtures thereof;
- (b) lactate selected from the group consisting of lactic acid, pharmaceutically acceptable salts of lactic acid, and mixtures thereof; and
- (c) a mixture of saturated and unsaturated
- 20 fatty acids wherein the fatty acids are those fatty acids required for the repair of cellular membranes and resuscitation of mammalian cells; and
- (3) (a) an antioxidant; and
- (b) a mixture of saturated and unsaturated
- 25 fatty acids wherein the fatty acids are those fatty acids required for the repair of cellular membranes and resuscitation of mammalian cells.

30 In a more preferred embodiment, the wound healing compositions for treating mammalian cells, preferably epidermal keratinocytes, may be selected from the group consisting of:

- (1) (a) pyruvate selected from the group consisting of pyruvic acid, pharmaceutically acceptable
- 35 salts of pyruvic acid, and mixtures thereof;
- (b) an antioxidant; and
- (c) a mixture of saturated and unsaturated fatty acids wherein the fatty acids are those fatty acids

required for the repair ^{of} cellular membranes and resuscitation of mammalian cells; and

5 (2) (a) pyruvate selected from the group consisting of pyruvic acid, pharmaceutically acceptable salts of pyruvic acid, and mixtures thereof;

(b) lactate selected from the group consisting of lactic acid, pharmaceutically acceptable salts of lactic acid, and mixtures thereof; and

10 (c) a mixture of saturated and unsaturated fatty acids wherein the fatty acids are those fatty acids required for the repair of cellular membranes and resuscitation of mammalian cells.

15 In a most preferred embodiment, the wound healing compositions for treating mammalian cells, preferably epidermal keratinocytes, comprise:

(a) pyruvate selected from the group consisting of pyruvic acid, pharmaceutically acceptable salts of pyruvic acid, and mixtures thereof;

20 (b) an antioxidant; and

(c) a mixture of saturated and unsaturated fatty acids wherein the fatty acids are those fatty acids required for the repair of cellular membranes and resuscitation of mammalian cells.

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In another preferred embodiment, the wound healing compositions for treating mammalian cells, preferably monocytes, comprise:

30 (a) lactate selected from the group consisting of lactic acid, pharmaceutically acceptable salts of lactic acid, and mixtures thereof;

(b) an antioxidant; and

35 (c) a mixture of saturated and unsaturated fatty acids wherein the fatty acids are those fatty acids required for the repair of cellular membranes and resuscitation of mammalian cells.

The present invention extends to methods for making the therapeutic wound healing compositions. In

general, a therapeutic composition is made by forming an admixture of the components of the composition. In one embodiment, a therapeutic composition is made by forming an admixture of (a) a pyruvate, (b) an antioxidant, and (c) a mixture of saturated and unsaturated fatty acids. In a second embodiment, a therapeutic composition is made by forming an admixture of (a) a pyruvate, (b) a lactate, and (c) a mixture of saturated and unsaturated fatty acids. In a third embodiment, a therapeutic composition is made by forming an admixture of (a) an antioxidant, and (b) a mixture of saturated and unsaturated fatty acids. In a fourth embodiment, a therapeutic composition is made by forming an admixture of (a) a lactate, (b) an antioxidant, and (c) a mixture of saturated and unsaturated fatty acids.

For some applications, the admixture may be formed in a solvent such as water. If necessary, the pH of the solvent is adjusted to a range from about 3.5 to about 8.0, and preferably from about 4.5 to about 7.5, and more preferably about 6.0 to about 7.4. The admixture is then sterile filtered. Other ingredients may also be incorporated into the therapeutic composition as dictated by the nature of the desired composition as well known by those having ordinary skill in the art. The ultimate therapeutic compositions are readily prepared using methods generally known in the pharmaceutical arts.

The present invention extends to methods for employing the therapeutic wound healing compositions. In general, a therapeutic composition is employed by contacting the therapeutic composition with a wound. In a specific embodiment, the invention is directed at a method for healing a wound in a mammal which comprises the steps of:

(A) providing a therapeutic wound healing composition which comprises:

(a) pyruvate selected from the group consisting of pyruvic acid, pharmaceutically acceptable salts of pyruvic acid, and mixtures thereof;

(b) an antioxidant; and

5 (c) a mixture of saturated and unsaturated fatty acids wherein the fatty acids are those fatty acids required for the repair of cellular membranes and resuscitation of mammalian cells; and

10 (B) contacting the wound healing composition with the wound.

In a first embodiment, the therapeutic compositions may be utilized by themselves to increase the proliferation and resuscitation rate of mammalian
15 cells. In a second embodiment, the therapeutic compositions may be combined with a medicament useful for treating wounds to form augmented wound healing compositions having an enhanced ability to further increase the proliferation and resuscitation rate of
20 mammalian cells.

In the first embodiment, the wound healing therapeutic compositions may be utilized by themselves in topical products to increase the proliferation and
25 resuscitation rate of mammalian cells. For example, the therapeutic compositions may be used in topical skin care products to treat wounds such as incisions and lacerations.

30 In the second embodiment, the therapeutic wound healing compositions of the present invention may be combined with medicaments useful for treating wounds to form augmented wound healing compositions. In this
35 embodiment, the combination of the therapeutic compositions of the present invention and the medicaments useful for treating wounds provides an augmented wound healing composition having an enhanced ability to increase the proliferation and resuscitation rate of mammalian cells. For example, the therapeutic

compositions of the present invention may be used in combination with medicaments useful for treating wounds such as anti-inflammatory agents, respiratory bursting inhibitors (lactic acid, adenosine), inhibitors of prostaglandin synthesis (ibuprofen, aspirin, indomethacin, meclofenomic acid, retinoic acid, padimate O, meclomen, oxybenzone), steroidal anti-inflammatory agents (corticosteroids including synthetic analogs), antibacterial agents, antimicrobial agents (neosporin ointment, silvadine), antiseptic agents, anesthetic agents (pramoxine hydrochloride, lidocaine, benzocaine), cell nutrient media, burn relief medications, sun burn medications, acne preparations, insect bite and sting medications, wound cleansers, wound dressings, scar reducing agents (vitamin E), immunostimulators (betafectin), and mixtures thereof, to further enhance the proliferation and resuscitation rate of mammalian cells. Preferably, the medicaments useful for treating wounds are selected from the group consisting of respiratory bursting inhibitors, inhibitors of prostaglandin synthesis, antimicrobial agents, cell nutrient media, scar reducing agents, and mixtures thereof. More preferably, the medicament useful for treating wounds is a cell nutrient medium.

A cell nutrient medium provides a complete diet of nutrients necessary for wound healing. The cell nutrient medium may be derived from animal, plant, and yeast sources. Typical cell nutrient media includes live yeast cell derivative, Eagles medium, and artificial serum. A preferred cell nutrient medium is live yeast cell derivative. Live yeast cell derivative supplies skin respiratory factor which acts by increasing the oxygen uptake of dermal tissues and facilitates collagen formation. Live yeast cell derivative generally contains numerous amino acids for collagen formation, mono- and disaccharides as carbon sources, vitamins, minerals, phosphorous containing compounds, nucleosides, nucleotides, and salts. In general, the amino acids

present in live yeast cell derivative include aspartic acid, glutamic acid, histidine, serine, glycine, alanine, arginine, tyrosine, valine, methionine, isoleucine, leucine, phenylalanine, and lysine. The coenzymes present in live yeast cell derivative include vitamin A, vitamin E, vitamin D₃, folic acid, pantothenic acid, niacinamide, vitamin B₁, vitamin B₂, vitamin B₆, and vitamin B₁₂. The cofactor type minerals present in live yeast cell derivative include calcium, copper, iron, magnesium, zinc, and phosphorus. A preferred tissue respiratory factor is Biodynes^R TRF, commercially available from Brooks Industries, Inc., South Plainfield, New Jersey. In general, the cell nutrient medium will be present in the therapeutic composition in an amount from about 0.01% to about 5%, preferably from about 0.1% to about 1%, and more preferably from about 0.2% to about 0.4%, by weight of the therapeutic composition.

In a specific embodiment, the invention is directed at an augmented wound healing composition which comprises:

(A) a therapeutic wound healing composition which comprises:

(a) pyruvate selected from the group consisting of pyruvic acid, pharmaceutically acceptable salts of pyruvic acid, and mixtures thereof;

(b) an antioxidant; and

(c) a mixture of saturated and unsaturated fatty acids wherein the fatty acids are those fatty acids required for the repair of cellular membranes and resuscitation of mammalian cells; and

(B) a medicament useful for treating wounds.

The present invention extends to methods for making the augmented wound healing compositions. In general, the augmented compositions are made by admixing the therapeutic wound healing composition with the medicament useful for treating wounds to prepare the augmented wound healing composition.

The present invention also extends to methods for employing the augmented wound healing compositions. In general, an augmented wound healing composition is employed by contacting the composition with a wound. In a specific embodiment, the invention is directed at a method for healing a wound in a mammal which comprises the steps of:

(A) providing a therapeutic wound healing composition which comprises:

(a) pyruvate selected from the group consisting of pyruvic acid, pharmaceutically acceptable salts of pyruvic acid, and mixtures thereof;

(b) an antioxidant; and

(c) a mixture of saturated and unsaturated fatty acids wherein the fatty acids are those fatty acids required for the repair of cellular membranes and resuscitation of mammalian cells;

(B) providing a medicament useful for treating wounds; and

(C) contacting the wound healing composition from step (A) and the medicament useful for treating wounds from step (B) concurrently with the wound.

The types of wounds which may be healed using the wound healing compositions and the augmented wound healing compositions of the present invention are those which result from an injury which causes epidermal damage such as incisions, wounds in which the skin is broken by a cutting instrument, and lacerations, wounds in which the skin is broken by a dull or blunt instrument. The therapeutic compositions may also be used to treat various dermatological disorders such as hyperkeratosis, photo-aging, burns, donor site wounds from skin transplants, ulcers (cutaneous, decubitis, venous stasis, and diabetic), psoriasis, skin rashes, and sunburn photoreactive processes. The topical therapeutic compositions may also be used orally in the form of a mouth wash or spray to protect and accelerate the healing

of injured oral tissue such as mouth sores and burns. The topical therapeutic compositions may further be used in ophthalmological preparations to treat wounds such as those which result from corneal ulcers, radialkeratotomy, corneal transplants, epikeratophakia and other surgically induced wounds in the eye. The topical therapeutic compositions may in addition be used in anorectal creams and suppositories to treat such conditions as pruritus ani, proctitis, anal fissures, and hemorrhoids. In a preferred embodiment, the therapeutic compositions are used to treat wounds such as incisions and lacerations.

Methods for healing a wound comprise topically administering the compositions of the present invention directly to a wound site to increase the healing rate of the wound. The composition is maintained in contact with the wound for a period of time sufficient to increase the proliferation and resuscitation rate of the cells.

Once prepared, the inventive therapeutic wound healing compositions and augmented wound healing compositions may be stored for future use or may be formulated in effective amounts with pharmaceutically acceptable carriers such as pharmaceutical appliances and topical vehicles (non-oral and oral) to prepare a wide variety of pharmaceutical compositions.

Examples of pharmaceutical appliances are sutures, staples, gauze, bandages, burn dressings, artificial skins, liposome or micell formulations, microcapsules, aqueous vehicles for soaking gauze dressings, and the like, and mixtures thereof. Non-oral topical compositions employ non-oral topical vehicles, such as oils, petrolatum bases, emulsions, lotions, creams, gels formulations, foams, ointments, sprays, salves, and films, which are intended to be applied to the skin or body cavity and are not intended to be taken by mouth. Oral topical compositions employ oral vehicles, such as mouthwashes, rinses, oral sprays,

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suspensions, bioadhesives, and dental gels, which are intended to be taken by mouth but are not intended to be ingested.

5 Bioadhesives are materials which adhere to live or freshly killed mucous membranes or skin tissues. Bioadhesives are generally water-swella-
ble but water-insoluble fibrous cross-linked materials. Bioadhesives generally comprise a mucoadhesive hydrogel such as a
10 polyacrylic acid cross linked by a polyhydroxy compound such as a carbohydrate (sugar, cyclitols) to form a substantially water-insoluble hydrogel. Other
bioadhesives include carboxymethylcellulose (CMC) and polycarbophils which are high molecular weight polymers
15 of acrylic acid such as Carbopol™ commercially available from BF Goodrich Company, Cleveland, Ohio. Bioadhesives are discussed in more detail in, for example, European
patent application no. 0410696A1, to Kellaway et al., and United States patent no. 4,615,697, issued to Robinson,
20 which disclosures are incorporated herein by reference.

 In one form of the invention, the therapeutic wound healing composition is incorporated into a pharmaceutical appliance which may be in the form of
25 sutures, staples, gauze, bandages, burn dressings, artificial skins, liposome or micell formulations, microcapsules, aqueous vehicles for soaking gauze dressings, and the like, and mixtures thereof. A variety
of traditional ingredients may optionally be included in
30 the pharmaceutical composition in effective amounts such as buffers, preservatives, tonicity adjusting agents, antioxidants, polymers for adjusting viscosity or for use as extenders, bioadhesives, and excipients, and the like. Specific illustrative examples of such traditional
35 ingredients include acetate and borate buffers; thimerosal, sorbic acid, methyl and propyl paraben and chlorobutanol preservatives; sodium chloride and sugars to adjust the tonicity; bioadhesives such as carboxymethylcellulose (CMC), Carbopol™, and

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polycarbophil; and excipients such as mannitol, lactose and sucrose.

In accordance with this invention, therapeutically effective amounts of the wound healing compositions of the present invention may be employed in the pharmaceutical appliance. These amounts are readily determined by those skilled in the art without the need for undue experimentation. The exact amount of the therapeutic composition employed is subject to such factors as the type and concentration of the therapeutic composition and the type of pharmaceutical appliance employed. Thus, the amount of therapeutic composition may be varied in order to obtain the result desired in the final product and such variations are within the capabilities of those skilled in the art without the need for undue experimentation. In a preferred embodiment, the pharmaceutical composition will comprise the therapeutic composition in an amount from about 0.1% to about 10%, by weight of the pharmaceutical composition. In a more preferred embodiment, the pharmaceutical composition will comprise the therapeutic composition in an amount from about 0.1% to about 8%, by weight of the pharmaceutical composition. In a most preferred embodiment, the pharmaceutical composition will comprise the therapeutic composition in an amount from about 0.1% to about 5%, by weight of the pharmaceutical composition.

The present invention extends to methods for making the pharmaceutical compositions. In general, a pharmaceutical composition is made by contacting a therapeutically effective amount of a wound healing composition with a pharmaceutical appliance and the other ingredients of the final desired pharmaceutical composition. The therapeutic composition may be in a solvent and may be absorbed onto a pharmaceutical appliance.

Other ingredients will usually be incorporated into the wound healing composition as dictated by the nature of the desired composition as well known by those having ordinary skill in the art. The ultimate pharmaceutical compositions are readily prepared using methods generally known in the pharmaceutical arts.

In another form of the invention, the therapeutic wound healing composition is incorporated into a non-oral topical vehicle which may be in the form of oils, petrolatum bases, emulsions, lotions, creams, gels formulations, foams, ointments, sprays, salves, and films, and the like. Typical non-toxic non-oral topical vehicles known in the pharmaceutical arts may be used.

The preferred non-oral topical vehicles are occlusive vehicles that minimize transepidermal water loss from the wound site. Minimizing transepidermal water loss promotes healing and reduces scarring. Preferred non-oral topical vehicles are petrolatum/mineral oil based products. Shark liver oil and cod liver oil may also be included to act as protectants to sooth and soften the tissues and minimize transepidermal water loss. Vitamin E may also be included to reduce scarring. Other non-oral topical vehicles such as emulsions and hydrogels may also be employed providing that the vehicles minimize transepidermal water loss. Other non-oral topical vehicles include water and pharmaceutically acceptable water-miscible organic solvents such as ethyl alcohol, isopropyl alcohol, propylene glycol, glycerin, and the like, and mixtures of these solvents.

The non-oral topical therapeutic compositions may also contain conventional additives employed in those products. Conventional additives include humectants, emollients, lubricants, stabilizers, dyes, and perfumes, providing the additives do not interfere with the therapeutic properties of the therapeutic composition.

Suitable humectants useful in the non-oral topical therapeutic compositions include glycerin, propylene glycol, polyethylene glycol, sorbitan, fructose, and the like, and mixtures thereof. Humectants, when employed, may be present in amounts from about 10% to about 20%, by weight of the topical therapeutic composition.

The coloring agents (colors, colorants) useful in the non-oral topical therapeutic composition are used in amounts effective to produce the desired color. These coloring agents include pigments which may be incorporated in amounts up to about 6% by weight of the non-oral topical therapeutic composition. A preferred pigment, titanium dioxide, may be incorporated in amounts up to about 2%, and preferably less than about 1%, by weight of the non-oral topical therapeutic composition. The coloring agents may also include natural food colors and dyes suitable for food, drug and cosmetic applications. These coloring agents are known as F.D. & C. dyes and lakes. The materials acceptable for the foregoing uses are preferably water-soluble. Illustrative nonlimiting examples include the indigoid dye known as F.D. & C. Blue No. 2, which is the disodium salt of 5,5-indigotindisulfonic acid. Similarly, the dye known as F.D. & C. Green No. 1 comprises a triphenylmethane dye and is the monosodium salt of 4-[4-(N-ethyl-p-sulfoniumbenzylamino) diphenylmethylene]-[1-(N-ethyl-N-p-sulfoniumbenzyl)-delta-2,5-cyclohexadieneimine]. A full recitation of all F.D. & C. coloring agents and their corresponding chemical structures may be found in the Kirk-Othmer Encyclopedia of Chemical Technology, 3rd Edition, in volume 5 at pages 857-884, which text is incorporated herein by reference.

In accordance with this invention, therapeutically effective amounts of the wound healing compositions of the present invention may be admixed with

a non-oral topical vehicle to form a topical therapeutic composition. These amounts are readily determined by those skilled in the art without the need for undue experimentation. In a preferred embodiment, the non-oral topical therapeutic compositions will comprise the therapeutic composition in an amount from about 0.1% to about 10% and a non-oral topical vehicle in a quantity sufficient to bring the total amount of composition to 100%, by weight of the non-oral topical therapeutic composition. In a more preferred embodiment, the non-oral topical therapeutic compositions will comprise the therapeutic composition in an amount from about 0.1% to about 10%, and in a most preferred embodiment, the non-oral topical therapeutic compositions will comprise the therapeutic composition in an amount from about 0.1% to about 8%, and a non-oral topical vehicle in a quantity sufficient to bring the total amount of composition to 100%, by weight of the non-oral topical therapeutic composition.

The present invention extends to methods for preparing the non-oral topical therapeutic compositions. In such a method, the non-oral topical therapeutic composition is prepared by admixing a therapeutically effective amount of the wound healing composition of the present invention and a non-oral topical vehicle. The final compositions are readily prepared using standard methods and apparatus generally known by those skilled in the pharmaceutical arts. The apparatus useful in accordance with the present invention comprises mixing apparatus well known in the pharmaceutical arts, and therefore the selection of the specific apparatus will be apparent to the artisan.

In another form of the invention, the wound healing composition is incorporated into an oral topical vehicle which may be in the form of a mouthwash, rinse, oral spray, suspension, dental gel, bioadhesive, and the like. Typical non-toxic oral vehicles known in the

pharmaceutical arts may be used in the present invention. The preferred oral vehicles are water, ethanol, and water-ethanol mixtures. The water-ethanol mixtures are generally employed in a weight ratio from about 1:1 to about 20:1, preferably from about 3:1 to about 20:1, and most preferably from about 3:1 to about 10:1, respectively. The pH value of the oral vehicle is generally from about 4 to about 7, and preferably from about 5 to about 6.5. An oral topical vehicle having a pH value below about 4 is generally irritating to the oral cavity and an oral vehicle having a pH value greater than about 7 generally results in an unpleasant mouth feel.

The oral topical therapeutic compositions may also contain conventional additives normally employed in those products. Conventional additives include a fluorine providing compound, a sweetening agent, a flavoring agent, a coloring agent, a humectant, a buffer, and an emulsifier, providing the additives do not interfere with the therapeutic properties of the therapeutic composition.

The coloring agents and humectants, and the amounts of these additives to be employed, set out above as useful in the non-oral topical therapeutic composition may be used in the oral topical therapeutic composition.

Fluorine providing compounds may be fully or slightly water soluble and are characterized by their ability to release fluoride ions or fluoride containing ions in water and by their lack of reaction with other components in the composition. Typical fluorine providing compounds are inorganic fluoride salts such as water-soluble alkali metal, alkaline earth metal, and heavy metal salts, for example, sodium fluoride, potassium fluoride, ammonium fluoride, cuprous fluoride, zinc fluoride, stannic fluoride, stannous fluoride, barium fluoride, sodium fluorosilicate, ammonium

fluorosilicate, sodium fluorozirconate, sodium monofluorophosphate, aluminum mono- and difluorophosphates and fluorinated sodium calcium pyrophosphate. Alkali metal fluorides, tin fluoride and monofluorophosphates, such as sodium and stannous fluoride, sodium monofluorophosphate and mixtures thereof, are preferred.

The amount of fluorine providing compound present in the present oral topical therapeutic composition is dependent upon the type of fluorine providing compound employed, the solubility of the fluorine compound, and the nature of the final oral therapeutic composition. The amount of fluorine providing compound used must be a nontoxic amount. In general, the fluorine providing compound when used will be present in an amount up to about 1%, preferably from about 0.001% to about 0.1%, and most preferably from about 0.001% to about 0.05%, by weight of the oral topical therapeutic composition.

When sweetening agents (sweeteners) are used, those sweeteners well known in the art, including both natural and artificial sweeteners, may be employed. The sweetening agent used may be selected from a wide range of materials including water-soluble sweetening agents, water-soluble artificial sweetening agents, water-soluble sweetening agents derived from naturally occurring water-soluble sweetening agents, dipeptide based sweetening agents, and protein based sweetening agents, including mixtures thereof. Without being limited to particular sweetening agents, representative categories and examples include:

(a) water-soluble sweetening agents such as monosaccharides, disaccharides and polysaccharides such as xylose, ribose, glucose (dextrose), mannose, galactose, fructose (levulose), sucrose (sugar), maltose, invert sugar (a mixture of fructose and glucose derived from sucrose), partially hydrolyzed starch, corn syrup

solids, dihydrochalcones, monellin, steviosides, and glycyrrhizin, and mixtures thereof;

(b) water-soluble artificial sweeteners such as soluble saccharin salts, i.e., sodium or calcium saccharin salts, cyclamate salts, the sodium, ammonium or calcium salt of 3,4-dihydro-6-methyl-1,2,3-oxathiazine-4-one-2,2-dioxide, the potassium salt of 3,4-dihydro-6-methyl-1,2,3-oxathiazine-4-one-2,2-dioxide (Acesulfame-K), the free acid form of saccharin, and the like;

10 (c) dipeptide based sweeteners, such as L-aspartic acid derived sweeteners, such as L-aspartyl-L-phenylalanine methyl ester (Aspartame) and materials described in United States patent no. 3,492,131, L-alpha-aspartyl-N-(2,2,4,4-tetramethyl-3-thietanyl)-D-alanine-
15 amide hydrate (Alitame), methyl esters of L-aspartyl-L-phenylglycerine and L-aspartyl-L-2,5-dihydrophenylglycine, L-aspartyl-2,5-dihydro-L-phenylalanine; L-aspartyl-L-(1-cyclohexen)-alanine, and the like;

(d) water-soluble sweeteners derived from naturally occurring water-soluble sweeteners, such as chlorinated derivatives of ordinary sugar (sucrose), e.g., chlorodeoxysugar derivatives such as derivatives of chlorodeoxysucrose or chlorodeoxygalactosucrose, known, for example, under the product designation of Sucralose; and

(e) protein based sweeteners such as thaumaococcus danielli (Thaumatococcus I and II).

In general, an effective amount of sweetening agent is utilized to provide the level of sweetness desired in the particular oral topical therapeutic composition, and this amount will vary with the sweetener selected and the final oral therapeutic product desired. The amount of sweetener normally present is in the range from about 0.0025% to about 90%, by weight of the oral topical therapeutic composition, depending upon the sweetener used. The exact range of amounts for each type of sweetener is well known in the art and is not the subject of the present invention.

The flavoring agents (flavors, flavorants) which may be used include those flavors known to the skilled artisan, such as natural and artificial flavors. Suitable flavoring agents include mints, such as peppermint, citrus flavors such as orange and lemon, artificial vanilla, cinnamon, various fruit flavors, both individual and mixed, and the like.

The amount of flavoring agent employed in the oral topical therapeutic composition is normally a matter of preference subject to such factors as the type of final oral therapeutic composition, the individual flavor employed, and the strength of flavor desired. Thus, the amount of flavoring may be varied in order to obtain the result desired in the final product and such variations are within the capabilities of those skilled in the art without the need for undue experimentation. The flavoring agents, when used, are generally utilized in amounts that may, for example, range in amounts from about 0.05% to about 6%, by weight of the oral topical therapeutic composition.

Suitable buffer solutions useful in the oral topical therapeutic compositions include citric acid-sodium citrate solution, phosphoric acid-sodium phosphate solution, and acetic acid-sodium acetate solution in amounts up to about 1%, and preferably from about 0.05% to about 0.5% by weight of the oral topical therapeutic composition.

In accordance with this invention, therapeutically effective amounts of the wound healing compositions of the present invention may be admixed with an oral topical vehicle to form a topical therapeutic composition. These amounts are readily determined by those skilled in the art without the need for undue experimentation. In a preferred embodiment, the oral topical therapeutic compositions will comprise the

therapeutic composition in an amount from about 0.1% to about 10% and a oral topical vehicle in a quantity sufficient to bring the total amount of composition to 100%, by weight of the oral topical therapeutic composition. In a more preferred embodiment, the oral topical therapeutic compositions will comprise the therapeutic composition in an amount from about 0.1% to about 10%, and in a most preferred embodiment, the oral topical therapeutic compositions will comprise the therapeutic composition in an amount from about 0.1% to about 8%, and a oral topical vehicle in a quantity sufficient to bring the total amount of composition to 100%, by weight of the oral topical therapeutic composition.

The present invention extends to methods for preparing the oral topical therapeutic compositions. In such a method, the oral topical therapeutic composition is prepared by admixing a therapeutically effective amount of the therapeutic composition of the present invention and an oral topical vehicle. The final compositions are readily prepared using standard methods and apparatus generally known by those skilled in the pharmaceutical arts. The apparatus useful in accordance with the present invention comprises mixing apparatus well known in the pharmaceutical arts, and therefore the selection of the specific apparatus will be apparent to the artisan.

In a preferred embodiment, an oral topical therapeutic composition is made by first dissolving coloring agents, sweetening agents, and similar additives in water. The therapeutic composition is then admixed with the aqueous solution. Then sufficient water or ethanol, or mixtures of water and ethanol, are added to the solution with mixing until the final solution volume is reached. In a more preferred embodiment, the therapeutic composition is added to the solution as the final ingredient. The final oral topical therapeutic

compositions are readily prepared using methods generally known in the pharmaceutical arts.

5 The oral therapeutic composition may also be in the form of dental gel. As used herein, the term "gel" means a solid or semisolid colloid which contains considerable quantities of water. The colloid particles in a gel are linked together in a coherent meshwork which immobilizes the water contained inside the meshwork.

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The dental gel compositions of the present invention may contain the conventional additives set out above for oral topical therapeutic compositions such as mouthwashes, rinses, oral sprays, and suspensions and, in addition, may contain additional additives such as a polishing agent, a desensitizing agent, and the like, providing the additional additives do not interfere with the therapeutic properties of the therapeutic composition.

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In a dental gel composition, the oral vehicle generally comprises water, typically in an amount from about 10% to about 90%, by weight of the dental gel composition. Polyethylene glycol, propylene glycol, glycerin, and mixtures thereof may also be present in the vehicle as humectants or binders in amounts from about 18% to about 30%, by weight of the dental gel composition. Particularly preferred oral vehicles comprise mixtures of water with polyethylene glycol or water with glycerin and polypropylene glycol.

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The dental gels of the present invention include a gelling agent (thickening agent) such as a natural or synthetic gum or gelatin. Gelling agents such as hydroxyethyl cellulose, methyl cellulose, glycerin, carboxypolymethylene, and gelatin and the like, and mixtures thereof may be used. Carboxymethylcellulose (CMC), Carbopol™, and polycarbophil may also be used. The preferred gelling agent is hydroxyethyl cellulose.

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Gelling agents may be used in amounts from about 0.5% to about 5%, and preferably from about 0.5% to about 2%, by weight of the dental gel composition.

5 The dental gel compositions of the present invention may also include a polishing agent. In clear gels, a polishing agent of colloidal silica and/or alkali metal aluminosilicate complexes is preferred since these materials have refractive indices close to the refractive
10 indices of the gelling systems commonly used in dental gels. In non-clear gels, a polishing agent of calcium carbonate or calcium dihydrate may be used. These polishing agents may be used in amounts up to about 75%, and preferably in amounts up to about 50%, by weight of
15 the dental gel composition.

 The dental gel may also contain a desensitizing agent such as a combination of citric acid and sodium citrate. Citric acid may be used in an amount from about
20 0.1% to about 3%, and preferably from about 0.2% to about 1%, by weight, and sodium citrate may be used in an amount from about 0.3% to about 9%, and preferably from about 0.6% to about 3%, by weight of the dental gel composition.

25 In accordance with this invention, therapeutically effective amounts of the wound healing compositions of the present invention may be admixed into the dental gel compositions. These amounts are readily
30 determined by those skilled in the art without the need for undue experimentation. In a preferred embodiment, the dental gel compositions will comprise the therapeutic composition in an amount from about 0.1% to about 10% and an oral topical vehicle in a quantity sufficient to bring
35 the total amount of composition to 100%, by weight of the dental gel composition. In a more preferred embodiment, the dental gel compositions will comprise the therapeutic composition in an amount from about 0.1% to about 5%, and in a most preferred embodiment, the dental gel

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compositions will comprise the therapeutic composition in an amount from about 0.1% to about 2%, and an oral topical vehicle in a quantity sufficient to bring the total amount of composition to 100%, by weight of the dental gel composition.

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The present invention extends to methods for preparing the therapeutic dental gel compositions. In such a method, the dental gel composition is prepared by admixing a therapeutically effective amount of the therapeutic composition of the present invention and an oral topical vehicle. The final compositions are readily prepared using methods generally known by those skilled in the dental and pharmaceutical arts. The apparatus useful in accordance with the present invention comprises mixing apparatus well known in the pharmaceutical arts, and therefore the selection of the specific apparatus will be apparent to the artisan.

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In a preferred embodiment, a therapeutic dental gel composition is made by first dispersing a gelling agent in a humectant or water, or a mixture of both, then admixing to the dispersion an aqueous solution of the water-soluble additives such as the fluorine providing compound, sweeteners and the like, then adding the polishing agent, and lastly admixing the flavoring agent and the therapeutic composition. The final gel mixture is then tubed or otherwise packaged. The liquids and solids in a gel product are proportioned to form a creamy or gelled mass which is extrudable from a pressurized container or from a collapsible tube. The final therapeutic compositions are readily prepared using methods generally known in the pharmaceutical arts.

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In a specific embodiment, the invention is directed at a wound healing pharmaceutical composition which comprises a pharmaceutically acceptable carrier and a therapeutically effective amount of a wound healing composition which comprises:

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(a) pyruvate selected from the group consisting of pyruvic acid, pharmaceutically acceptable salts of pyruvic acid, and mixtures thereof;

(b) an antioxidant; and

5 (c) a mixture of saturated and unsaturated fatty acids wherein the fatty acids are those fatty acids required for the repair of cellular membranes and resuscitation of mammalian cells.

10 The pharmaceutically acceptable carrier may be selected from the group consisting of pharmaceutical appliances and topical vehicles. Preferably, the topical vehicle is an occlusive vehicle.

15 In another specific embodiment, the invention is directed at a method for preparing a pharmaceutical composition for increasing the proliferation and resuscitation rate of mammalian cells, which comprises the steps of:

20 (A) providing a therapeutically effective amount of a wound healing composition which comprises:

(a) pyruvate selected from the group consisting of pyruvic acid, pharmaceutically acceptable salts of pyruvic acid, and mixtures thereof;

25 (b) an antioxidant; and

(c) a mixture of saturated and unsaturated fatty acids wherein the fatty acids are those fatty acids required for the repair of cellular membranes and resuscitation of mammalian cells;

30 (B) providing a pharmaceutically acceptable carrier; and

(C) admixing the wound healing composition from step (A) and the pharmaceutically acceptable carrier from step (B) to form a pharmaceutical composition.

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Throughout this application, various publications have been referenced. The disclosures in these publications are incorporated herein by reference in order to more fully describe the state of the art.

The present invention is further illustrated by the following examples which are not intended to limit the effective scope of the claims. All parts and percentages in the examples and throughout the specification and claims are by weight of the final composition unless otherwise specified.

EXAMPLES 1-5

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These examples demonstrate a comparison of the wound healing abilities of the therapeutic wound healing compositions of the present invention versus conventional wound healing compositions.

15

The wound healing compositions of Examples A-D were prepared having the compositions set out in Table 1.

Table 1

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Ingredient	Examples			
	A	B	C	D
	Prep. H TM			
sodium pyruvate	--	2%	--	--
vitamin E	--	1%	--	--
chicken fat	--	2%	--	--
LYCD	2000 U*	2400 U	2400 U	--
shark liver oil	3%*	3%	3%	--
petrolatum	in	64%	66.5%	68%
mineral oil	amounts	22.53%	25.03%	26.8%
paraffin	totaling	5%	5%	5%
emulsifier	100%*	0.2%	0.2%	0.2%

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* These components are present in Preparation HTM

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Wound healing composition A was commercially available Preparation H™. Wound healing composition B was a petrolatum base formulation containing live yeast cell derivative, shark oil, and a mixture of sodium pyruvate, vitamin E, and chicken fat. Wound healing composition C was a petrolatum base formulation containing live yeast cell derivative and shark oil. Wound healing composition D was a petrolatum base formulation only.

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Wound healing studies were carried out using hairless mice (SKR-1, Charles River) 6-8 weeks in age. One group of mice were untreated as a control group and were referred to as Example E. In each group there were 6 mice for evaluation at either day 3 or day 7 for a total number of 60 animals in the study. The mice were anesthetized with ether and a midline 3 cm full thickness longitudinal incision was made with a number 10 scalpel blade. Incisions were closed using steel clips at 1 cm intervals. Formulations A-D set out above were applied in a randomized blinded study to the wounds on day 0 at 2 hours following wounding and reapplied at 24 hour intervals during the 7 days of the study. The wounds were examined daily and scored on a basis of 0-5 for closure on each day of the study, with a score of 5 representing the wound best healed.

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The animals were sacrificed on day 3 and day 7 using cervical dislocation. The dorsal skin including the incision was dissected without the subcutaneous tissue. The skin was placed in neutral buffered formalin and subsequently sectioned and stained with hematoxylin and eosin. The wounds were examined microscopically and representative tissue sections were photographed.

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On each day of the experiment, the score and rank order of the formulations for closure of wounds and speed of healing were as follows:

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B (5) >> D (4) >> C (2) >/= E, Control (2) > A (1)

Photographs of the wounded mice on day 4 are set out in FIGURES 1 and 2.

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FIGURES 1 and 2 show that Formulation B, which was a petrolatum base formulation containing live yeast cell derivative, shark oil, and a mixture of sodium pyruvate, vitamin E, and chicken fat, was a significantly better wound healing agent than the other formulations. These results are supported by the subjective grading of the wound closures and the speed of healing on each day (1-7) of the experiment as well as on the objective histological examination of tissue sections to measure the extent of inflammatory cell infiltrate within the wound and the extent of epithelialization at the wound edges. The final result was that less scar tissue was present at day 7 on the mice treated with Formulation B.

20

Formulation D, which was a white petrolatum formulation only, was judged to be significantly more effective to promote healing than either Formulation C, which was a petrolatum base formulation containing shark liver oil and live yeast cell derivative, or Formulation A, which was Preparation HTM. The superior ability of Formulation D over Formulation C to improve healing may result from a delay in the healing process caused when the live yeast cell derivative is depleted and the cells shift to an alternative nutrient source. The presence of the mixture of sodium pyruvate, vitamin E, and chicken fat in Formulation B apparently offsets the depletion of the live yeast cell derivative.

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Formulation C, which was a petrolatum base formulation containing live yeast cell derivative and shark oil, was judged comparable to the control (untreated wound) in speed of wound closure and extent of healing. Formulation A, which was Preparation HTM, appeared to be the least effective healing formulation by

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both subjective grading of wound healing and by objective examination of tissue sections. The superior ability of Formulation D and Formulation C over Formulation A to improve healing may be due to their ability to act as an occlusive wound dressing that prevents transepidermal water loss and thus promotes healing and wound closure. The poor ability of Formulation A to improve healing may be due to the potential cytotoxicity of phenylmercuric nitrate present in Preparation HTM as a preservative.

10

These results show that the wound healing compositions of the present invention which comprise a mixture of sodium pyruvate, vitamin E, and chicken fat increase the proliferation and resuscitation rate of mammalian cells. The wound healing compositions mediate low levels of oxygen in the initial stages of healing to suppress oxidative damage and higher levels of oxygen in the later stages of healing to promote collagen formation.

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The invention being thus described, it will be obvious that the same may be varied in many ways. Such variations are not to be regarded as a departure from the spirit and scope of the invention and all such modifications are intended to be included within the scope of the following claims.

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I claim:

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1. A therapeutic wound healing composition which comprises:

5 (a) pyruvate selected from the group consisting of pyruvic acid, pharmaceutically acceptable salts of pyruvic acid, and mixtures thereof;

(b) an antioxidant; and

10 (c) a mixture of saturated and unsaturated fatty acids wherein the fatty acids are those fatty acids required for the repair of cellular membranes and resuscitation of mammalian cells.

2. The composition according to claim 1,
15 wherein the mammalian cells comprise epidermal keratinocytes.

3. The composition according to claim 1,
20 wherein the pyruvate is selected from the group consisting of pyruvic acid, sodium pyruvate, potassium pyruvate, magnesium pyruvate, calcium pyruvate, zinc pyruvate, manganese pyruvate, and mixtures thereof.

4. The composition according to claim 3,
25 wherein the pyruvate is sodium pyruvate.

5. The composition according to claim 1,
wherein the antioxidant is selected from the group consisting of retinol, 3, 4-didehydroretinol, alpha-carotene, beta-carotene, gamma-carotene, delta-carotene,
30 ascorbic acid, alpha-tocopherol, beta-tocopherol, gamma-tocopherol, delta-tocopherol, and mixtures thereof.

6. The composition according to claim 5,
35 wherein the antioxidant is alpha-tocopherol.

7. The composition according to claim 1,
wherein the mixture of saturated and unsaturated fatty acids comprises animal and vegetable fats and waxes.

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8. The composition according to claim 7, wherein the mixture of saturated and unsaturated fatty acids comprises human fat, chicken fat, cow fat, sheep fat, horse fat, pig fat, and whale fat.

9. The composition according to claim 8, wherein the mixture of saturated and unsaturated fatty acids comprises lauric acid, myristic acid, myristoleic acid, pentadecanoic acid, palmitic acid, palmitoleic acid, margaric acid, margaroleic acid, stearic, oleic acid, linoleic acid, linolenic acid, arachidic acid, and gaddoleic acid.

10. The composition according to claim 1, wherein pyruvate is present in the therapeutic composition in an amount from about 10% to about 50%, by weight of the therapeutic composition.

11. The composition according to claim 1, wherein the antioxidant is present in the therapeutic composition in an amount from about 10% to about 50%, by weight of the therapeutic composition.

12. The composition according to claim 1, wherein the mixture of saturated and unsaturated fatty acids is present in the therapeutic composition in an amount from about 10% to about 50%, by weight of the therapeutic composition.

13. A method for preparing a therapeutic wound healing composition which comprises the steps of admixing the following ingredients:

(a) pyruvate selected from the group consisting of pyruvic acid, pharmaceutically acceptable salts of pyruvic acid, and mixtures thereof;

(b) an antioxidant; and

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(c) a mixture of saturated and unsaturated fatty acids wherein the fatty acids are those fatty acids required for the repair of cellular membranes and resuscitation of mammalian cells.

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14. A method for healing a wound in a mammal which comprises the steps of:

(A) providing a therapeutic wound healing composition which comprises:

10 (a) pyruvate selected from the group consisting of pyruvic acid, pharmaceutically acceptable salts of pyruvic acid, and mixtures thereof;

(b) an antioxidant; and

15 (c) a mixture of saturated and unsaturated fatty acids wherein the fatty acids are those fatty acids required for the repair of cellular membranes and resuscitation of mammalian cells; and

(B) contacting the wound healing composition with the wound.

20

15. An augmented wound healing composition which comprises:

(A) a therapeutic wound healing composition which comprises:

25 (a) pyruvate selected from the group consisting of pyruvic acid, pharmaceutically acceptable salts of pyruvic acid, and mixtures thereof;

(b) an antioxidant; and

30 (c) a mixture of saturated and unsaturated fatty acids wherein the fatty acids are those fatty acids required for the repair of cellular membranes and resuscitation of mammalian cells; and

(B) a medicament useful for treating wounds.

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16. The augmented wound healing composition according to claim 15, wherein the medicament useful for treating wounds is selected from the group consisting of anti-inflammatory agents, respiratory bursting inhibitors, inhibitors of prostaglandin synthesis, antibacterial agents, antimicrobial agents, antiseptic agents, anesthetic agents, cell nutrient media, burn relief medications, sun burn medications, acne preparations, insect bite and sting medications, wound cleansers, wound dressings, scar reducing agents, immunostimulators, and mixtures thereof.

17. A method for preparing an augmented wound healing composition which comprises the steps of:

(A) providing a therapeutic wound healing composition which comprises:

(a) pyruvate selected from the group consisting of pyruvic acid, pharmaceutically acceptable salts of pyruvic acid, and mixtures thereof;

(b) an antioxidant; and

(c) a mixture of saturated and unsaturated fatty acids wherein the fatty acids are those fatty acids required for the repair of cellular membranes and resuscitation of mammalian cells;

(B) providing a medicament useful for treating wounds; and

(C) admixing the wound healing composition from step (A) with the medicament useful for treating wounds from step (B) to prepare the augmented wound healing composition.

18. A method for healing a wound in a mammal which comprises the steps of:

(A) providing a therapeutic wound healing composition which comprises:

(a) pyruvate selected from the group consisting of pyruvic acid, pharmaceutically acceptable salts of pyruvic acid, and mixtures thereof;

(b) an antioxidant; and

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(c) a mixture of saturated and unsaturated fatty acids wherein the fatty acids are those fatty acids required for the repair of cellular membranes and resuscitation of mammalian cells;

5 (B) providing a medicament useful for treating wounds; and

(C) contacting the wound healing composition from step (A) and the medicament useful for treating wounds from step (B) concurrently with the wound.

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19. A wound healing pharmaceutical composition which comprises a pharmaceutically acceptable carrier and a therapeutically effective amount of a wound healing composition which comprises:

15 (a) pyruvate selected from the group consisting of pyruvic acid, pharmaceutically acceptable salts of pyruvic acid, and mixtures thereof;

(b) an antioxidant; and

20 (c) a mixture of saturated and unsaturated fatty acids wherein the fatty acids are those fatty acids required for the repair of cellular membranes and resuscitation of mammalian cells.

25 20. The pharmaceutical composition according to claim 19, wherein the pharmaceutically acceptable carrier is a pharmaceutical appliance.

30 21. The pharmaceutical composition according to claim 19, wherein the pharmaceutically acceptable carrier is a bioadhesive.

22. The pharmaceutical composition according to claim 19, wherein the pharmaceutically acceptable carrier is a topical vehicle.

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23. The pharmaceutical composition according to claim 22, wherein the topical vehicle is an occlusive vehicle.

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24. The pharmaceutical composition according to claim 19, wherein the wound healing composition is present in the pharmaceutical composition in an amount from about 0.1% to about 10%, by weight.

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25. A method for preparing a wound healing pharmaceutical composition which comprises the steps of:

(A) providing a therapeutically effective amount of a wound healing composition which comprises:

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(a) pyruvate selected from the group consisting of pyruvic acid, pharmaceutically acceptable salts of pyruvic acid, and mixtures thereof;

(b) an antioxidant; and

15 (c) a mixture of saturated and unsaturated fatty acids wherein the fatty acids are those fatty acids required for the repair of cellular membranes and resuscitation of mammalian cells;

(B) providing a pharmaceutically acceptable carrier; and

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(C) admixing the wound healing composition from step (A) and the pharmaceutically acceptable carrier from step (B) to form a pharmaceutical composition.

INTERNATIONAL SEARCH REPORT

International Application No

PCT/US 92/08787

I. CLASSIFICATION OF SUBJECT MATTER (If several classification symbols apply, indicate all) ⁹		
According to International Patent Classification (IPC) or to both National Classification and IPC		
Int. Cl. 5	A 61 K 31/20	A 61 K 31/375 A 61 K 31/355 //
(A 61 K 31/20	A 61 K 31:19	A 61 K 31:07) (A 61 K 31/20,
II. FIELDS SEARCHED		
Minimum Documentation Searched ⁷		
Classification System	Classification Symbols	
Int. Cl. 5	A 61 K	
Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched ⁸		
III. DOCUMENTS CONSIDERED TO BE RELEVANT⁹		
Category ⁹	Citation of Document, ¹¹ with Indication, where appropriate, of the relevant passages ¹²	Relevant to Claim No. ¹³
A	Biological Abstracts, vol. 75, 1983, Biological Abstract Inc., (Philadelphia, PA, US), H. WOLTERS et al.: "Radiation effects on membranes: 3. The effect of X irradiation on survival of mammalian cells substituted by polyunsaturated fatty acids", abstract no. 68500, see abstract ---	
A	EP, A, 0347056 (EFAMOL HOLDINGS PLC) 20 December 1989, see claim 4 ---	
A	DE, A, 3719097 (U. FRATZER) 9 June 1988, see abstract -----	
<div style="display: flex; justify-content: space-between;"> <div style="width: 45%;"> <p>⁹ Special categories of cited documents: ¹⁰</p> <p>"A" document defining the general state of the art which is not considered to be of particular relevance</p> <p>"E" earlier document but published on or after the international filing date</p> <p>"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)</p> <p>"O" document referring to an oral disclosure, use, exhibition or other means</p> <p>"P" document published prior to the international filing date but later than the priority date claimed</p> </div> <div style="width: 45%;"> <p>"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention</p> <p>"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step</p> <p>"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.</p> <p>"&" document member of the same patent family</p> </div> </div>		
IV. CERTIFICATION		
Date of the Actual Completion of the International Search	Date of Mailing of this International Search Report	
11-12-1992	25.01.93	
International Searching Authority	Signature of Authorized Officer	
EUROPEAN PATENT OFFICE	<i>J. M. S. 4000</i>	

INTERNATIONAL SEARCH REPORT

International Application No. PCT/US 92/08787 -2-

I. CLASSIFICATION OF SUBJECT MATTER (If several classification symbols apply, indicate all)		
According to International Patent Classification (IPC) or to both National Classification and IPC Int.Cl.5 31:19, 31:015), (A 61 K 31/375, 31:20, 31:19), (A 61 K 31/355, 31:20, 31:19)		
II. FIELDS SEARCHED		
Minimum Documentation Searched ⁷		
Classification System	Classification Symbols	
Int.Cl.5		
Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched ⁸		
III. DOCUMENTS CONSIDERED TO BE RELEVANT⁹		
Category ¹⁰	Citation of Document, ¹¹ with indication, where appropriate, of the relevant passages ¹²	Relevant to Claim No. ¹³
<div style="display: flex; justify-content: space-between;"> <div style="width: 45%;"> <p>¹⁰ Special categories of cited documents: 10</p> <p>"A" document defining the general state of the art which is not considered to be of particular relevance</p> <p>"E" earlier document but published on or after the international filing date</p> <p>"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)</p> <p>"O" document referring to an oral disclosure, use, exhibition or other means</p> <p>"P" document published prior to the international filing date but later than the priority date claimed</p> </div> <div style="width: 45%;"> <p>"F" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention</p> <p>"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step</p> <p>"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.</p> <p>"G" document member of the same patent family</p> </div> </div>		
IV. CERTIFICATION		
Date of the Actual Completion of the International Search	Date of Mailing of this International Search Report	
	25.01.93	
International Searching Authority	Signature of Authorized Officer	
EUROPEAN PATENT OFFICE		

Mme Dagmar FRANK

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 92/08787

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:
ALTHOUGH CLAIMS 14, 18 ARE DIRECTED TO A METHOD OF TREATMENT OF (DIAGNOSTIC METHOD PRACTISED ON) THE HUMAN/ANIMAL BODY THE SEARCH HAS BEEN CARRIED OUT AND BASED ON THE ALLEGED EFFECTS OF THE COMPOUND/COMPOSITION.
2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
IN VIEW OF THE LARGE NUMBER OF COMPOUNDS WHICH ARE DEFINED BY THE WORDING OF THE CLAIMS THE SEARCH HAS BEEN PERFORMED ON THE GENERAL IDEA AND COMPOUNDS MENTIONED IN THE EXAMPLES OF THE DESCRIPTION(PCT. ART 6; GUIDELINES:PART B; CHAPTER II.7 LAST SENTENCE AND CHAPTER III.;3.7)
3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest.
- ☐ No protest accompanied the payment of additional search fees.

**ANNEX TO THE INTERNATIONAL SEARCH REPORT
ON INTERNATIONAL PATENT APPLICATION NO.**

US 9208787
SA 65839

This annex lists the patent family members relating to the patent documents cited in the above-mentioned international search report. The members are as contained in the European Patent Office EDP file on 05/01/93. The European Patent Office is in no way liable for these particulars which are merely given for the purpose of information.

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
EP-A- 0347056	20-12-89	AU-B- 618814	09-01-92
		AU-A- 3597489	14-12-89
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